Thermo SCIENTIFIC

PRODUCT INFORMATION

Agarase

Pub. No. MAN0012011
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#EO0461 **100 U**

Lot: _ **Expiry Date: _**

Concentration: 0.5 U/µL

Store at -20 °C

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For Research Use Only. Not for use in diagnostic procedures.

Description

Agarase specifically digests the agarose polysaccharide core into oligosaccharides (1). Agarase allows for gentle yet efficient recovery of DNA or RNA fragments from low melting point agarose. The recovered nucleic acids can be directly used for amplification, cloning, sequencing, etc.

Applications

Quantitative recovery of DNA or RNA from low melting point (LM) agarose gels (*see* protocol on back page).

Source

E.coli cells with a plasmid containing the gene encoding β-agarase from *Pseudomonas atlantica.*

Molecular Weight

32.7 kDa monomer.

Definition of Activity Unit

One unit of the enzyme completely degrades 100 µL (approx. 100 mg) of molten 1% low melting point agarose in 30 min at 42 °C.

Enzyme activity is assayed in 1X TBE buffer:
89 mM Tris base, 89 mM boric acid, 2 mM EDTA.

Storage Buffer

The enzyme is supplied in: 50 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 0.1% (v/v) Triton X-100 and 50% (v/v) glycerol.

Inhibition and Inactivation

- Inhibitors: ethidium bromide at concentrations higher than 5 µg/mL, transition metal ions.
- Inactivated by heating at 70 °C for 10 min.

Note

- Activity of agarase in different buffers (in comparison to activity in assay buffer (1X TBE)):

1X TBE (90 mM Tris-borate, 2 mM EDTA, pH 8.3)	100%
1X TAE (40 mM Tris-acetate, 1 mM EDTA, pH 8.5)	120%
1X TPE (90 mM Tris-phosphate, 2 mM EDTA, pH 7.7)	120%
1X Bis-Tris (50 mM Bis-Tris-HCl, 1 mM EDTA, pH 6.5)	150%

- Conventional agarose is not suitable for digestion by agarase.

CERTIFICATE OF ANALYSIS

Endodeoxyribonuclease Assay

Incubation of supercoiled plasmid DNA with Agarase.

Ribonuclease Assay

Incubation of RNA transcript with Agarase.

Labeled Oligonucleotide (LO) Assay

Incubation of single stranded and double stranded radiolabeled oligonucleotides with Agarase.

Quality authorized by:  Jurgita Zilinskiene

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Protocol for recovery of DNA from LM agarose gels

1. Perform electrophoresis of DNA in a low melting point (LM) agarose (#R0801) gel prepared in TAE (#B49), 0.5X TBE, TBE (#B52) or TPE buffer. Stain the gel with ethidium bromide.
2. Cut out the desired DNA band from the agarose gel with a clean scalpel under UV light*. Cut out only as much agarose as necessary. (The bottom of the excised agarose slice is free of DNA and should be removed).
3. Determine the weight of the slice. To facilitate melting, cut gel slices larger than 200 mg into smaller pieces.
4. Incubate the tube at 70 °C for approx. 10 min. Ensure that the agarose is completely molten.
5. Transfer the tube to a 42 °C water bath and equilibrate for 5 min.
6. Add 1 U of Agarase (#EO0461) per 100 mg (approx. 100 µL) of molten 1% LM agarose. Increase the amount of enzyme proportionally for higher percentage agarose, gently mix and incubate at 42 °C for 30 min.
7. Add ammonium acetate** to a 2.5 M final concentration, chill on ice for 5 min.
8. Centrifuge at 10,000 rpm for 10 min to pellet undigested carbohydrates. Transfer the supernatant to a clean tube.
9. Add 2.5 volumes of ethanol or 0.8 volume of isopropanol, mix gently and incubate at room temperature for 1 h. If DNA fragments are smaller than 500 kb or if the DNA concentration is lower than 0.05 µg/mL, incubate at room temperature for 2 h.
10. Centrifuge at 10,000 rpm for 15 min, remove supernatant and dry the pellet. Resuspend the pellet in TE or another appropriate buffer for subsequent manipulation.

Note

* For subcloning of gel-purified DNA fragments, care should be taken to avoid DNA damage with UV light. minimize the UV exposure to a few seconds or keep the gel on a glass or plastic plate during UV illumination. Alternatively, visible dyes can be included in standard agarose gels to visualize DNA bands in ambient light (1, 2).

** Ammonium acetate is recommended rather than other salts to avoid co-precipitation of oligosacharides with DNA.

- The procedure typically recovers 90% of DNA from the gel.
- T4 Polynucleotide Kinase is inhibited by ammonium ions, therefore use 3 M Sodium Acetate, #R1181, (0.3 M final concentration) if T4 Polynucleotide Kinase will be used in downstream applications.
- Large DNA fragments (>30 kb) require delicate handling to avoid mechanical shearing. After digestion with Agarase (step 6), centrifuge at maximum speed for 10 min to pellet undigested carbohydrates. Remove oligosacharides and agarase by dialysis or carry out subsequent manipulations with DNA in the digested agarose solution.

Reference

1. Yaphe, W., The use of agarase from *Pseudomonas atlantica* in the identification of agar in marine algae (*Rhodophyceae*), Can. J. Microbiol., 3, 987-993, 1957.